Autoregulation of Human Retinal Blood Flow

An Investigation With Laser Doppler Velocimetry

C. E. Riva, J. E. Grunwald, and D. L. Petrig

The effect of acute changes in mean retinal perfusion pressure, \( \bar{P} \) (\( \frac{3}{2} \) of mean brachial artery blood pressure minus IOP), on retinal volumetric blood flow rate, \( Q \), was investigated in normal volunteers. Changes in \( Q \) were determined from \( Q = k \cdot V_{\text{max}} \cdot D^2 \), where \( V_{\text{max}} \) is the center line red blood cell velocity measured from temporal veins by laser Doppler velocimetry, \( D \) is the vessel diameter obtained by monochromatic fundus photography, and \( k \) is a constant of proportionality. A suction cup was used to induce step changes in IOP and, consequently, in \( \bar{P} \). The magnitude of the steps ranged from 10–32 mmHg. During the first 30 sec after a step decrease in \( \bar{P} \), \( V_{\text{max}} \) and \( Q \) were significantly smaller than at rest by an amount proportional to the decrease in \( \bar{P} \). Thereafter, \( V_{\text{max}} \) and \( Q \) increased markedly towards their values at rest, although \( \bar{P} \) changed comparatively little during this period of time. Time constant of the corresponding decrease in vascular resistance, \( R(t) = \frac{\bar{P}(t)}{Q(t)} \), was approximately 45 sec. There was no significant change in \( D \) during elevated IOP. Removal of the cup induced an immediate step increase in \( \bar{P} \), \( V_{\text{max}} \), \( D \), \( Q \), and \( R \). Thereafter, \( V_{\text{max}} \), \( D \), \( Q \), and \( R \) returned to their values at rest (time constant of the change in \( R \) was about 30 sec), while \( \bar{P} \) remained nearly constant. The rapid change in vascular resistance following a step decrease and increase in \( \bar{P} \) can be attributed to an active process that attempts to maintain blood flow close to normal, in spite of changes in perfusion pressure (autoregulation). The results show, however, that autoregulation, although present at \( \bar{P} \)s as low as 10 mmHg (IOP \( \approx \) 42 mmHg) is fully effective only if \( \bar{P} \) is not lowered by more than 50% (IOP not above some value between 27 and 30 mmHg). Invest Ophthalmol Vis Sci 27:1706–1712, 1986

The effect of raised intraocular pressure (IOP) on retinal blood flow has been investigated in a number of papers. \(^1\)–\(^9\) Studies performed in anesthetized animals using a variety of techniques have shown conflicting results. Some have found retinal blood flow to be efficiently regulated over a large range of raised IOP. \(^5\)–\(^10\) Others have reported that blood flow decreases when the IOP increases. \(^1\)

In contrast to animal studies, investigations in the intact human eye are scant. Techniques based on the blue field entoptic phenomenon have demonstrated the presence of a regulatory process operating with a time constant of approximately 1/2 min. \(^7\) They have shown that leukocyte velocity and, presumably, blood flow in retinal capillaries remain normal under conditions of acutely increased IOPs up to approximately 30 mmHg. \(^7\),\(^9\)

Whereas the techniques based on the blue field phenomenon provide information on blood flow in the macular region of the retina, laser Doppler velocimetry (LDV), combined with vessel diameter measurements, enables the determination of blood flow through large segments of the retina. \(^11\) Thus, both methods complement each other, and applying them may demonstrate whether the changes in blood flow in the large vessels parallel those in the microvessels of the central retina.

In this paper, we present the results of an investigation of the effect of acute elevations of IOP on retinal volumetric blood flow rate in the intact human eye using LDV and monochromatic fundus photography. The results are compared to those obtained previously in humans with techniques based on the blue field entoptic phenomenon.

Materials and Methods

Measurements were performed in one eye of each of seven healthy volunteers, with ages ranging from 21–47 years (33 \( \pm \) 9 years, mean \( \pm \) 1 SD). Subjects had no history of systemic hypertension, and their ocular examination was normal. They were familiar with the technique, cooperative, and had good target fixation. Informed consent was obtained from each subject after the procedures had been explained. IOP at rest, IOP\(_{\text{rest}}\).
recorded by Goldmann applanation tonometry ranged from 11–16 mmHg (13 ± 1.5 mmHg). Mean brachial artery blood pressure, P_b, measured by sphygmomanometry and calculated as P_b = P_dia + \frac{1}{2}(P_{syst} - P_{dia}) was between 70 and 84 mmHg (mean 78 ± 5 mmHg). P_{syst} and P_{dia} are the systolic and diastolic brachial artery pressures, respectively. Mean retinal perfusion pressure, defined as \( P = \frac{1}{2}P_b - IOP \), ranged from 34–44 mmHg (mean 39 ± 3.5 mmHg). The factor \( \frac{1}{2} \) accounts for the drop in blood pressure between brachial and ophthalmic artery.

**Change in the IOP**

After dilatation of the pupil with tropicamide 1%, a drop of 0.5% proparacaine hydrochloride (Alcon Laboratories, Inc, Fort Worth, Texas) was applied to the eye, and IOP_{rest} was measured. A Langham pressure cup was then placed on the temporal sclera, and the IOP was raised stepwise by increasing rapidly the negative pressure to the cup. For the LDV measurements, each of the following three negative cup pressures were applied in each subject on a different day: 80, 110, and 140 mmHg. The cup pressure was not altered until the cup was removed. The IOP was measured by Goldmann applanation tonometry at about 2, 4, 6, and 8 min. For this measurement, the subject rotated himself by 90° from a position facing the LDV camera to a position facing a Haag-Streit slit lamp. After 8–10 min of suction, the cup was quickly removed and the IOP measured every 2 min thereafter for 6–10 min. The systemic pressure was then remeasured.

For the investigation of the effect of raised IOP on vessel diameter, the negative cup pressure was raised quickly to 140 mmHg, kept at this level for approximately 10 min, and then quickly dropped to zero. IOP was measured every 2 min after application of the cup, and the systemic pressure was taken at the beginning and the end of the experiment. This experiment was performed on a different day.

**Blood Velocity**

Bidirectional LDV measurements were obtained, as previously described, to determine the maximum or center line velocity, V_{max}, of red blood cells in a main temporal retinal vein. The cutoff frequencies of the Doppler shift power spectra were obtained by computer analysis. The analyzed spectra were sampled from the recorded LDV signals by an examiner who was unaware of the IOP at which the measurements were obtained. V_{max} at IOP_{rest}, V_{max,rest}, was calculated from an average of 15 pairs of spectra, each pair recorded during about 300 msec, whereas, at elevated IOP, V_{max} was determined from three to five successive pairs of spectra. The location of each measurement was within two disc diameters from the center of the disc, and was marked on a color fundus photograph for referencing diameter measurements. Sites close to venous junctions or arteriovenous crossings were avoided.

**Vessel Diameter**

The diameter, D, of a main temporal vein at the site of LDV recordings and of a main temporal artery at a site located within two disc diameters from the center of the disc were determined from monochromatic (\( \lambda = 0.57 \mu m \)) fundus photographs. The photographs were taken with a fundus camera at IOP_{rest}, during about 8 min of application of the 140 mmHg cup pressure and after removal of the cup. The negatives were projected onto a screen, and D was measured with a caliper by an observer masked with regard to the actual sequence of the photographs.

**Volumetric Blood Flow Rate**

Venous volumetric blood flow rate, Q, was determined from the relationship:

\[
Q = k \cdot V_{max} \cdot D^2, \quad \text{(Equation 1)}
\]

where k is a constant of proportionality between \( V_{mean} \), the mean velocity of whole blood, and \( V_{max} \). In this study, the value of k is unimportant, since we are interested only in the determination of the changes in \( V_{mean} \) and Q, which are induced by the changes in IOP and P. The validity of the proportionality between \( V_{mean} \) and \( V_{max} \) is discussed later (see “Discussion”).

**Vascular Resistance**

From \( \bar{P} \) and Q, the vascular resistance, R, can be determined from the relation \( R = \frac{P}{Q} \) using Equation 1: \( R = \frac{\bar{P}}{Q/k \cdot V_{max} \cdot D^2} \). The relative change in R with time was calculated as:

\[
\frac{R(t)}{R_{rest}} = \frac{\bar{P}(t) \cdot V_{max,rest} \cdot D_{rest}^2}{\bar{P}_{rest} \cdot V_{max(t)} \cdot D^2(t)} \quad \text{(Equation 2)}
\]

All results are presented in terms of mean ± one half of its 95% confidence interval.

**Results**

**Effect of Cup Pressure on IOP and Mean Perfusion Pressure**

Table 1 summarizes the group average of IOP_{rest} and \( \bar{P}_{rest} \) and IOP(0) and \( \bar{P}(0) \). IOP(0) and \( \bar{P}(0) \), which correspond to the IOP and \( \bar{P} \) present immediately after the application of the negative cup pressure, were obtained by linear extrapolation of all IOPs measured during the time of application of the cup. While the suction pressure was maintained constant, the IOP decreased continuously. The rate of decrease,
Table 1. Effect of different levels of suction pressure on IOP and mean perfusion pressure, $\bar{P}$

<table>
<thead>
<tr>
<th>Number of Experiments</th>
<th>$IOP_{rest}$ (mmHg)</th>
<th>$\bar{P}_{rest}$ (mmHg)</th>
<th>Suction Pressure (mmHg)</th>
<th>$IOP(0)$ (mmHg)</th>
<th>$\bar{P}(0)$ (mmHg)</th>
<th>$\gamma$ (mmHg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>14.6 ± 1.4*</td>
<td>39 ± 8*</td>
<td>80</td>
<td>30 ± 3*</td>
<td>24 ± 4*</td>
<td>0.9 ± 2*</td>
</tr>
<tr>
<td>7</td>
<td>15.1 ± 1.2</td>
<td>39 ± 3.5</td>
<td>110</td>
<td>35 ± 4</td>
<td>19 ± 6</td>
<td>1.2 ± 2</td>
</tr>
<tr>
<td>10</td>
<td>11.8 ± 0.9</td>
<td>39 ± 2.5</td>
<td>140</td>
<td>41 ± 2</td>
<td>10 ± 4</td>
<td>1.4 ± 2</td>
</tr>
</tbody>
</table>

* One-half the 95% confidence interval of the mean.

$IOP_{rest}$, $\bar{P}_{rest}$ = Group average IOP and mean perfusion pressure at rest; $IOP(0)$, $\bar{P}(0)$ = Group average IOP and mean perfusion pressure immediately after application of the suction pressure to the cup. These values were estimated from a linear fit of the IOP and $\bar{P}$ values measured at approximately 2, 4, 6, and 8 min after application of the cup at time 0; $\gamma$ = Group average rate of decrease of the IOP with time while the suction pressure was left unchanged.

$$\gamma$$ (mmHg/min), which was estimated from a linear fit of the IOP values, increased significantly ($t$-test, $P < 0.05$) with the magnitude of the suction pressure and, therefore, with that of the step increase of the IOP.

Removal of the suction pressure caused the IOP to drop stepwise from an average of 27 mmHg to an average of 5 mmHg, and $\bar{P}$ to rise stepwise from an average of 25 mmHg to an average of 47 mmHg. The latter represents an average step increase in $\bar{P}$ of approximately 88%. Thereafter, the IOP rose and $\bar{P}$ decreased at an average rate of about 0.5 mmHg/min.

**Time Course of Arterial and Venous $D$ and of Venous $V_{max}$**

At $IOP_{rest}$, the group average diameter of the arteries was 115 ± 12 μm, with a range from 98–133 μm. Average vein diameter was 147 ± 21 μm, with a range from 117–168 μm. Average $V_{max}$ at $IOP_{rest}$ was 1.72 ± 0.39 cm/sec, with a range from 1.31–2.45 cm/sec.

The time course of the group average venous $D(t)/D_{rest}$ is represented in Figure 1. Although this ratio is smaller than unity during the first 2 min of elevated IOP, neither the individual average values nor the average of all of them was significantly different from $D_{rest}$, although the IOP was still an average of 7 mmHg below the average $IOP_{rest}$ of approximately 14 mmHg.

Removal of the cup caused a significant increase in venous $D$ ($P < 0.05$). After about 4 min, however, venous $D$ was no longer significantly different from $D_{rest}$, although the IOP was still an average of 7 mmHg below the average $IOP_{rest}$ of approximately 14 mmHg.

Group average arterial $D$ was not significantly different from its value at rest during the application of the cup, but was significantly greater (3.1%, $P < 0.05$) during the first 30 sec after removal of the cup.

The time course of $V_{max}$ after the step elevation in IOP could be characterized by a rapid and significant ($P < 0.01$) decrease occurring immediately at the application of the cup, followed by an increase of $V_{max}$ towards $V_{max,rest}$ (Fig. 2). The rate of this increase was faster than expected from the increase in $\bar{P}$. At 5–8 min, $V_{max}$ reached a value which differed from $V_{max,rest}$.

**Fig. 1.** Time course of the group average relative venous diameter in response to an acute increase and decrease of the IOP. The bar represents the 95% confidence interval of the mean. For some of the points in both graphs, the error bars were too small to be drawn.

**Fig. 2.** Representative time course of $V_{max}$ following an acute increase in IOP (decrease in mean perfusion pressure, $\bar{P}$). The bar represents the 95% confidence interval of the mean. The suction cup was applied at time zero.
Table 2. $V_{\text{max}}/V_{\text{max,rest}}$ ratio measured between 5 and 8 min after application of the cup as a function of IOP

<table>
<thead>
<tr>
<th>Number of Experiments</th>
<th>IOP (mmHg)</th>
<th>$V_{\text{max}}/V_{\text{max,rest}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>23–26</td>
<td>0.96 ± .12*</td>
</tr>
<tr>
<td>16</td>
<td>27–30</td>
<td>0.74 ± .08†</td>
</tr>
<tr>
<td>22</td>
<td>31–34</td>
<td>0.77 ± .05†</td>
</tr>
</tbody>
</table>

* One-half the 95% confidence interval of the mean.
† Significantly different from unity ($P < 0.05$).

by an amount that depended upon the IOP at this time (Table 2). However, group average $V_{\text{max}}$ was significantly different from $V_{\text{max,rest}}$ (paired t-test, $P < 0.05$) only when the IOP was in the range 27–30 mmHg, corresponding to a $P$ in the range 22–25 mmHg; i.e., a decrease of 36–43% from $P_{\text{rest}}$.

Figure 3 shows the individual values of $V_{\text{max}}/V_{\text{max,rest}}$ versus $P$ measured during the first 30 sec after application of the cup. There is a significant correlation between these two quantities ($P < 0.01$), and the regression line (a) intersects the velocity axis at a value which is very close to zero, although significantly different from it ($P < 0.05$). The regression line (b) of $V_{\text{max}}/V_{\text{max,rest}}$ on $P$ for the measurements obtained between 3 and 8 min of elevated IOP is also significant ($P < 0.001$). Compared to line a, however, line b is markedly shifted towards greater values of $V_{\text{max}}/V_{\text{max,rest}}$.

Figure 4 shows group averages of $V_{\text{max}}/V_{\text{max,rest}}$, from which we have estimated the closed loop gain, $G$, of the autoregulatory system (Fig. 4, inset). $G$ is defined by the ratio $(Q_b - Q_a)/(1 - Q_a)$, where $Q_a$ and $Q_b$ are the respective blood flows immediately after application of the cup and after autoregulation has occurred. Since $D$ did not change significantly during the period of raised IOP, this ratio can be approximated by $(V_{\text{max,b}} - V_{\text{max,a}})/(1 - V_{\text{max,a}})$.

In four subjects (11 experiments), $V_{\text{max}}$ could be measured during a few minutes after removal of the cup. In eight experiments, the operator could realign the fundus camera quickly enough to take measurements within the first 30 sec. Figure 5 shows the results obtained from one of these experiments. During the first 30 sec, group average $V_{\text{max}}$ was 1.6 ± 5 times greater than $V_{\text{max,rest}}$, and, since the average diameter was greater than normal by an average of about 10%, $Q$ was approximately twice its value at rest. Thereafter, $V_{\text{max}}$ decreased quickly towards the baseline, with a time constant $\tau_{\text{off}}$ of about 29 sec. $\tau_{\text{off}}$ was determined from a three-parameter exponential least square fit ($P < 0.01$) of the $V_{\text{max}}$ values using a simplex algorithm.

The function was defined by the equation $V_{\text{max}} = a_1 + a_2 e^{-t/\tau_{\text{off}}}$, with $a_1$ and $a_2$ being constants (Fig. 6).

**Time Course of the Vascular Resistance**

The calculated $R(t)/R_{\text{rest}}$ values were grouped according to time, and, for each group, an average value was calculated. A plot of average $R(t)/R_{\text{rest}}$ as a function of time during raised IOP and after removal of the cup is shown in Figure 7. After a transient increase immediately after application of the cup, the vascular resistance decreased to reach a constant level at approximately 2 min. About 63% of the total decrease in resistance that took place during the period of raised IOP had occurred at 45 sec. When the cup was removed, this resistance returned to its value at rest within 2–4 min, with a time constant of about 30 sec.

![Fig. 3. Plot of $V_{\text{max}}/V_{\text{max,rest}}$ versus mean perfusion pressure, $P$.](image)

This ratio was calculated from $V_{\text{max}}$ values obtained during the first 30 sec (●) and between 3 and 8 minutes (○) after application of the cup. There is a significant correlation between the two quantities measured during the first 30 sec (regression line a) and between 3 and 8 min (regression line b) ($P < 0.01$ and $P < 0.001$, respectively). The bar along the ordinate represents ± 1 SD for the average value of $V_{\text{max}}/V_{\text{max,rest}}$ at $P = 0$, which was determined from the regression analysis (line a). Line c corresponds to values of $V_{\text{max}}/V_{\text{max,rest}}$ that would be expected from a passive system.

![Fig. 4. Comparison between the average value $V_{\text{max}}/V_{\text{max,rest}}$ measured during the first 30 sec (●) and between 3 and 8 min (○) of elevated IOP as a function of $P$.](image)

The horizontal and vertical bar represent the 95% confidence interval of the mean of $P$ and $V_{\text{max}}/V_{\text{max,rest}}$. In the inset, the closed loop gain, $G$, of the autoregulation process has been plotted as a function of $P$. 

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Fig. 5. Representative time course of $V_{max}$ following removal of the suction cup after approximately 9 min of elevated IOP. Also shown are the values of $V_{max}$ at rest and at 7 and 8 min of elevated IOP, and immediately after the cup was removed. Each data point represents an average of $V_{max}$ values measured during a period of approximately 5 sec. The bar represents the 95% confidence interval of the mean.

Mean brachial artery blood pressure and pulse rate did not vary significantly during the whole experiment. In the fundus photographs of three subjects chosen at random, the distance between landmark points on the fundus, such as the edge of the disc and a vessel bifurcation, was measured. Neither at elevated IOP nor after removal of the suction cup have we detected a significant change in linear magnification of the retina on the film.

Discussion

Laser Doppler velocimetry and monochromatic fundus photography were applied to investigate the effect of step changes in mean perfusion pressure, $\bar{P}$, on the maximum or centerline red blood cell velocity, $V_{max}$, in retinal veins and the vessel diameter, $D$. We have chosen to determine $V_{max}$ in veins and not in arteries to avoid the tedious calculations required in the determination of the time averaged velocity of pulsatile arterial blood velocity, and also because, in general, venous $V_{max}$ shows less variability than arterial $V_{max}$.11

In determining $Q$ from $V_{max}$ and $D$, we have assumed that the mean velocity of whole blood, $V_{mean}$, is proportional to $V_{max}$; i.e., $V_{mean} = k \cdot V_{max}$, with $k$ independent of diameter and velocity. Although this relationship has not been tested in vivo, in vitro measurements have shown its validity for the flow of whole blood in glass tubes with diameters ranging from 10–160 $\mu$m and velocities ranging from 0.09–2.13 cm/sec.14 These diameters and velocities are comparable to those measured in this study. In addition, we have also assumed that changes in vessel diameter accurately represent changes in the cross-section of the vessel, as is the case if the shape of the vessels does not change over the range of perfusion pressure tested. For the veins to change their shape, the transmural pressure must become smaller than zero,15 a condition which does not occur in the range of IOPs studied here, since an increase in IOP is accompanied by an equal rise in intracocular venous pressure.16

As shown in Figure 3, relative $V_{max}$ measured during the first 30 sec after application of the cup was smaller than $V_{max, rest}$ by an amount that increased with the magnitude of the step decrease in $P$. This result is in agreement with previous observations in pig eyes.4 This figure also shows that the regression line $V_{max}/V_{max, rest}$ vs $\bar{P}$ intersects the $\bar{P}$ axis at a $\bar{P}$ value which is very close to zero. These two findings are consistent with an initially passive behaviour of the retinal vascular system during the first 30 sec of decreased perfusion pressure. In contrast, the marked increase in venous $V_{max}$ which can be observed thereafter, and the marked upward shift of the regression line determined from $V_{max}$ values measured between 3 and 8 min of raised IOP (Figs. 4, 5), cannot be accounted for by the rela-
tively slow increase in $P$ during this period of time. Rather, it must be the result of an active process which tends to normalize blood flow, in spite of the reduced perfusion pressure, by means of decreasing retinal vascular resistance (Fig. 7). The time constant of the decrease in vascular resistance of approximately 45 sec is within the range of the values obtained in a previous investigation.

The average increase in $V_{max}$ (60%), $D$ ($\approx 10\%$), and $Q$ ($\approx 95\%$) occurring immediately after the removal of the cup corresponds closely to the value expected from an 88% increase in $P$. The subsequent rapid decrease in $V_{max}$, $D$, and $Q$ must again be due to an active autoregulatory process, since this decrease takes place at nearly constant perfusion pressure. The time constant of this response, approximately 30 sec, appears to be somewhat shorter than that of the response to a decrease in $P$. More measurements are needed to determine whether these two time constants differ significantly from each other and, thus, represent a different type of control.

The mechanism that alters the vascular resistance in response to changes in perfusion pressure must act on the wall of the smaller arterioles to account for the marked change in vascular resistance that takes place in the presence of insignificant or minimal (3.1%) changes in the diameter of the large arteries where LDV measurements were performed. Since the changes in vascular resistance take between 1 and 2 min for completion, this mechanism most probably includes metabolic processes. However, one cannot exclude a myogenic process in which the response would be limited by the slowness of contraction of the vascular smooth muscle itself. A central neural process is excluded by the lack of intrinsic sympathetic innervation of the retinal vessels.

Autoregulation has been defined, in its strict sense, as the maintenance of constant blood flow, in spite of changes in perfusion pressure. In this sense, the data shown in Table 2 demonstrates that the retinal circulation autoregulates up to some IOP (called previously $IOP_{max}$), between 27 and 30 mmHg, since, in this range, $V_{max}$ measured between 5 and 8 min of increased IOP is not significantly smaller than $V_{max,rest}$. This value is similar to the IOP$_{max}$ value of approximately 30 mmHg, which was obtained in a previous study. One of these studies estimated, however, that 30 mmHg would represent an upper limit for $IOP_{max}$. Autoregulation has also been defined, in a wider sense, as the tendency of blood flow to remain constant in face of changes in perfusion pressure. Using this definition, we can conclude from Figure 4 that autoregulation is still effective at $P$ as low as 10 mmHg ($IOP \approx 42$ mmHg). Indeed, at this $P$, relative $V_{max}$ and, consequently, relative $Q$ have twice the value they would have in the absence of regulation. This corresponds to a closed loop gain of the regulatory mechanism of approximately 1, down from a value of 3 at a $P$ of 30 mmHg ($IOP \approx 22$ mmHg). In other words, at $P = 10$ mmHg, the regulatory response is three times less efficient than at $P = 30$ mmHg.

Previous studies of retinal blood flow autoregulation in eyes with open-angle glaucoma have demonstrated that, in these eyes, $IOP_{max}$ was lower than normal, and suggested that the reactive hyperemia was absent during the first few minutes after removal of the cup. It would be expected, therefore, that the relationship between blood flow and perfusion pressure is different from normal in these eyes, and could result in a faster reduction of the gain of the regulatory mechanism with increased IOP. At what time this occurs during the development of glaucoma remains to be investigated.

**Key words:** intraocular pressure, perfusion pressure, retinal blood flow, laser Doppler velocimetry, retinal hemodynamics, autoregulation, vascular resistance

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